Organization TC1600 Bldg./Room REMSEN

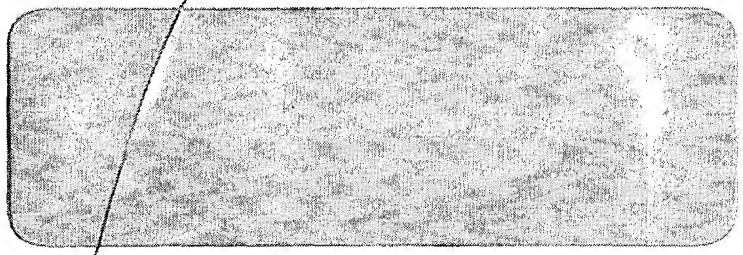
U. S. DEPARTMENT OF COMMERCE
COMMISSIONER FOR PATENTS
P.O. BOX 1450
ALEXANDRIA, VA 22313-1450
IF UNDELIVERABLE RETURN IN TEN DAYS

OFFICIAL BUSINESS

U.S. OFFICIAL MAIL
PENALTY FOR PRIVATE USE $300

UNITED STATES POSTAGE
PITNEY BOWES
02 1A
0004202245
$ 01.75⁹
APR 12 2004
MAILED FROM ZIPCODE 22202

AN EQUAL OPPORTUNITY EMPLOYER



RETURNED TO SENDER
NOT DELIVERABLE AS ADDRESSED
UNABLE TO FORWARD
NEW YORK, NY 10036

RECEIVED
APR 20 2004
TECH CENTER 1600/2900

UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
09/528,644	03/20/2000	Lars Thim	3951.224-US	5698

7590 04/12/2004

Steve T Zelson Esq
Novo Nordisk of North America Inc
405 Lexington Avenue
Suite 6400
New York, NY 10017

EXAMINER	
ROMEO, DAVID S	

ART UNIT	PAPER NUMBER
1647	

DATE MAILED: 04/12/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/528,644	THIM ET AL.	
	Examiner	Art Unit	
	David S Romeo	1647	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *11 December 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *27-33,36 and 40-65* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *27-33,36 and 40-65* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☒ Claim(s) *27-33,36 and 40-65* are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

DETAILED ACTION

The amendment filed 12/11/2003 has been entered. Claims 27-33, 36, 40-65 are pending.

Applicant's election of the species a homolog of SEQ ID NO: 1 wherein the homolog is identical to SEQ ID NO: 1 except for two amino acid substitutions, wherein the amino acid substitutions are in the first trefoil domain in the paper filed 12/11/2003 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).

Claims 47, 49, 55 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected species, there being no allowable generic or linking claim. Election was made **without** traverse in the paper filed 12/11/2003.

Maintained Formal Matters, Objections, and/or Rejections:

Claims 27-33, 36, 42-59 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a glycosylated polypeptide comprising the amino acid sequence of SEQ ID NO: 1, does not reasonably provide enablement for "homologue" useful for the treatment of ulcers. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention commensurate in scope with these claims.

In addition the specification does not provide an assay for measuring usefulness in the treatment of ulcers. It is unclear what endpoint is intended by the limitation "useful in the treatment of ulcers."

It is further noted that a nucleic acid molecule that hybridizes to a nucleic acid molecule encoding SEQ ID NO: 1 is antisense to a nucleic acid molecule encoding SEQ ID NO: 1 and does not encode anything resembling SEQ ID NO: 1. The specification lacks guidance for, and working examples of, using such variant polypeptides for the treatment of ulcers.

Applicants argue that the present specification teaches how to make SEQ ID NO: 1 and refer to Playford et al. Applicant's arguments have been fully considered but they are not persuasive. A disclosure of SEQ ID NO: 1 in the present Applicant or in Playford is not commensurate with claimed "homologue." Furthermore, while a specification need not disclose what is well known in the art, that rule does not excuse an applicant from providing a complete disclosure. It is the specification, not the knowledge of one skilled in the art, that must supply the novel aspects of an invention in order to constitute adequate enablement. Although certain claims are limited to the substitution of only two amino acids, the specification does not provide an assay for measuring usefulness in the treatment of ulcers and it is unclear what endpoint is intended by the limitation "useful in the treatment of ulcers."

Applicants argue that Bowie concludes that proteins are surprisingly tolerant of amino acid substitutions and that the test for undue experimentation is not merely quantitative. Applicant's arguments have been fully considered but they are not persuasive. Except for the deletion or creation of glycosylation sites, the instant specification does not identify those amino acid residues in the amino acid sequence of SEQ ID NO: 1 which are tolerant of amino acid

substitutions. To practice the instant invention in a manner consistent with the breadth of the claims would not require just a repetition of work that is described in the instant application but a substantial inventive contribution on the part of a practitioner which would involve the determination of those amino acid residues in the amino acid sequence of SEQ ID NO: 1 which are required for the functional and structural integrity of that protein. It is this additional characterization of that single disclosed, naturally occurring protein that is required in order to obtain the functional and structural data needed to permit one to produce a "homologue" which meets both the structural and functional requirements of the instant claims that constitutes undue experimentation.

Applicant has taken the position that 35 U.S.C. § 112, first paragraph, permits an artisan to present claims of essentially limitless breadth so long as the specification provides one with the ability to test any particular embodiment which is encompassed by the material limitations of a claim and thereby distinguish between those embodiments which meet the functional limitations from those that don't. However, the issue here is the breadth of the claims in light of the predictability of the art as determined by the number of working examples, the skill level of the artisan and the guidance provide by the instant specification and the prior art of record. An inventor should be allowed to dominate future patentable inventions of others where those inventions were based in some way on his teachings, since such improvements, while unobvious from his teachings, are still within his contribution, since improvement was made possible by his work; however, he must not be permitted to achieve this dominance by claims which are insufficiently supported and, hence, not in compliance with first paragraph of 35 U.S.C. 112; that paragraph requires that scope of claims must bear a reasonable correlation to scope of

enablement provided by specification to persons of ordinary skill in the art; in cases involving predictable factors, such as mechanical or electrical elements, a single embodiment provides broad enablement in the sense that, once imagined, other embodiments can be made without difficulty and their performance characteristics predicted by resort to known scientific laws; in cases involving unpredictable factors, such as most chemical reactions and physiological activity, scope of enablement varies inversely with degree of unpredictability of factors involved. It is noted that there is not a single example in the instant specification, working or prophetic, of a "homologue" whose amino acid sequence deviates from nature other than the creation or deletion of a glycosylation site. The instant specification provides no working examples and no guidance that would permit an artisan to practice the invention commensurate with the scope of the instant claims. The first paragraph of 35 U.S.C. § 112 requires that the breadth of the claims must be based upon the predictability of the claimed subject matter and not on some standard of trial and error. Unless one has a reasonable expectation that any one material embodiment of the claimed invention would be more likely than not to function in the manner disclosed or the instant specification provides sufficient guidance to permit one to identify those embodiments which are more likely to work than not without actually making and testing them then the instant application does not support the breadth of the claims.

Claims 27-33, 36, 40-65 are rejected under 35 U.S.C. 112, second paragraph, over the recitation of "high stringency conditions." Applicants argue that the phrase would have a clear and definite meaning to one of skill in the art. Applicants arguments have been fully considered

but they are not persuasive. The present specification's reference to Sambrook is merely exemplary, and is not intended to limit the definition of "high stringency conditions" in any way.

Double Patenting

Claims 27-33, 36, 40, 41, 42-65 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-8, 10-13 of U.S. Patent No. 5,783,416 (a6). It is acknowledged that Applicants intend to submit a terminal disclaimer when the present application is allowable.

New Formal Matters, Objections, and/or Rejections:

Claim Rejections - 35 USC § 103

Claims 27-30, 32, 40, 42, 43, 45, 46, 48, 50, 51, 60-62 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tomasetto (2, cited by Applicants) in view of Podolsky (A), Alberts (u6), Hitzeman (x6), and Lodish (w6).

Tomasetto discloses the cDNA and deduced amino acid sequence of a human spasmolytic polypeptide (hSP) (paragraph bridging pages 409-410; Figure 5). The encoded protein contains a putative signal sequence, amino acids 1-24 (Figure 5). The amino acid sequence of the encoded protein minus the putative signal peptide is identical to Applicants' SEQ ID NO:1, as indicated below (Qy = Applicants' SEQ ID NO: 1) (Db = hSP):

```
RESULT    1
ENTRY             S12371     #type fragment
TITLE             spasmolytic protein 1 precursor - human (fragment)
ALTERNATE_NAMES   trefoil factor 2
ORGANISM          #formal_name Homo sapiens #common_name man
DATE              21-Nov-1993 #sequence_revision 24-May-1996 #text_change
                    18-Sep-1998
ACCESSIONS        S12371
REFERENCE         S12371
   #authors       Tomasetto, C.; Rio, M.C.; Gautier, C.; Wolf, C.; Hareuveni,
                    M.; Chambon, P.; Lathe, R.
   #journal       EMBO J. (1990) 9:407-414
   #title         hSP, the domain-duplicated homolog of pS2 protein, is
                    co-expressed with pS2 in stomach but not in breast
                    carcinoma.
   #cross-references MUID:90151615
```

```
   #accession     S12371
      ##molecule_type mRNA
      ##residues       1-130 ##label TOM
      ##cross-references EMBL:X51698; NID:g36558; PID:g36559
GENETICS
   #gene          GDB:TFF2; SML1
      ##cross-references GDB:128989; OMIM:182590
   #map_position 21q22.3
FUNCTION
   #description  inhibits gastrointestinal motility and gastric acid secretion
CLASSIFICATION   #superfamily spasmolytic protein; trefoil homology
KEYWORDS         duplication; hormone; pancreas
FEATURE
   1-24               #domain signal sequence (fragment) #status predicted
                        #label SIG\
   25-130             #product spasmolytic protein #status predicted #label
                        MAT\
   32-73              #domain trefoil homology #label TRF1\
   82-122             #domain trefoil homology #label TRF2\
   30-128,32-59,43-58,
   53-70,82-108,
   92-107,102-119     #disulfide_bonds #status predicted
SUMMARY          #length 130  #checksum 8997

  Query Match          100.0%;  Score 857;  DB 1;  Length 130;
  Best Local Similarity 100.0%;  Pred. No. 6.82e-177;
  Matches   106;  Conservative    0;  Mismatches   0;  Indels   0;  Gaps   0;

Db      25 EKPSPCQCSRLSPHNRTNCGFPGITSDQCFDNGCCFDSSVTGVPWCFHPLPKQESDQCVM 84
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Qy       1 EKPSPCQCSRLSPHNRTNCGFPGITSDQCFDNGCCFDSSVTGVPWCFHPLPKQESDQCVM 60

Db      85 EVSDRRNCGYPGISPEECASRKCCFSNFIFEVPWCFFPNSVEDCHY 130
           |||||||||||||||||||||||||||||||||||||||||||||||
Qy      61 EVSDRRNCGYPGISPEECASRKCCFSNFIFEVPWCFFPNSVEDCHY 106.
```

hSP "has six disulfide bonds ... and 9-12" because hSP and SEQ ID NO: 1 are identical in structure or composition. Furthermore, a chemical composition and its properties are inseparable. hSP is encoded by a nucleic acid sequence that is at least 60% homologous to a nucleic acid sequence that encodes SEQ ID NO: 1 because the amino acid sequence of hSP and SEQ ID NO: 1 are identical. The nucleic acid sequence encoding hSP also encodes SEQ ID NO: 1. Therefore, the nucleic acid sequence encoding hSP would hybridize under high stringency conditions to complement of the nucleic acid sequence encoding SEQ ID NO: 1. hSP contains the amino acid sequence Asn-X-Ser/Thr, wherein X is any amino acid, at amino acid residues 39-41, which is a classic N-linked glycosylation site. Amino acid residues 39-41 of hSP correspond to amino acids 15-17 of Applicants' SEQ ID NO:1. Tomasetto discloses strong conservation of primary structure between PSP, mSP and hSP which suggest that these three proteins fulfill similar biological functions (page 412, column 2, full paragraph 4). Tomasetto does not teach an isolated hSP polypeptide which is in N-glycosylated form, in the sense that Tomasetto does not anticipate the claimed invention.

Podolsky teaches rat intestinal trefoil factor (rITF), which has significant homology to pS2 and porcine pancreatic spasmolytic peptide (PSP). PSP and pS2 are both thought to fold into a characteristic structure referred to as a trefoil. A trefoil structure consists of three loops formed by three disulfide bonds. pS2 is thought to include one trefoil, and PSP is thought to include two trefoils. The region of rITF which is most similar to PSP and pS2 includes six cysteines all of which are in the same position as the cysteines which make up the trefoil in pS2. Five of these six cysteines are in the same position as the cysteines which form the amino terminal trefoil of PSP. Paragraph bridging columns 4-5. The isolated hITF gene can be cloned into a mammalian expression vector for protein expression. This vector can be used to express the protein in COS cells, CHO cells, or mouse fibroblasts. The gene may also be cloned into a vector for expression in Drosophila cells using the baculovirus expression system. Column 8, lines 40-51. Podolsky also discloses a therapeutic composition that includes an intestinal trefoil factor and a pharmacologically acceptable carrier (column 1, lines 39, 41, 58-60; claim 3). Podolsky does not teach an isolated hSP polypeptide which is in N-glycosylated form, in the sense that Podolsky does not anticipate the claimed invention.

However, it would have been obvious to one of ordinary skill in the art at the time of Applicants' invention to clone a nucleic acid molecule encoding hSP, as taught by Tomasetto, and to modify that teaching by cloning the hSP nucleic acid molecule into a mammalian expression vector and express the nucleic acid molecule in mammalian cells, as taught by Podolsky, with a reasonable expectation of success. One of ordinary skill in the art would be motivated to combine these teachings because expression in mammalian cells is useful for the expression of trefoil proteins. Recombinant expression of hSP in mammalian cells would have

the advantage of providing a large amount of readily purified hSP. In so doing one of ordinary skill in the art would obtain an isolated hSP polypeptide which is in N-glycosylated form, glycosylated at an Asn present at position 15, comprising a glycosylated side chain comprising at least one hexose unit, wherein the glycosylated side chain comprises at least one mannose unit, wherein the glycosylated form comprises two units of GlcNAc, containing 39 amino acids in the first trefoil domain, containing 38 amino acids in the second trefoil domain. The invention is prima facie obvious over the prior art.

Alberts, Hitzeman, and Lodish are cited as evidence of what was in the public's possession before applicant's invention.

Alberts teaches that most of the soluble proteins that are secreted are glycoproteins (page 589, penultimate paragraph).

Hitzeman teaches N-linked glycosylation at the amino acid sequence Asn-X-Ser/Thr wherein X is any amino acid (page 436, full paragraph 2).

Lodish teaches that most secreted proteins are glycosylated (page 699, column 2, full paragraph 3), that in all N-linked oligosaccharides N-acetylglucosamine is linked to Asn (page 700, column 1, first full sentence), that N-linked oligosaccharides always contain mannose as well as N-acetylglucosamine (page 700, column 1, third sentence), that two GlcNAc and three Man are always found in *N*-linked oligosaccharides (page 701, legend to Figure 16-27), and that N-linked oligosaccharides can have as many as 60 mannose residues in yeast (page 701, legend to Figure 16-27).

Claims 27, 36, 40, 41 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tomasetto (2, cited by Applicants) in view of Podolsky (A), Alberts (u6), Hitzeman (x6), and Lodish (w6), as applied to claims 27 and 40 above, and further in view of Jorgensen.

Tomasetto in view of Podolsky, Alberts, Hitzeman, and Lodish teach an isolated hSP polypeptide which is in N-glycosylated form. Tomasetto in view of Podolsky, Alberts, Hitzeman, and Lodish do not teach a pharmaceutical composition comprising an isolated hSP polypeptide which is in N-glycosylated form and a pharmaceutically acceptable carrier.

Jorgensen teaches a pharmaceutical composition comprising PSP and a pharmaceutically acceptable carrier (page 232, full paragraphs 1 and 2; page 233, full paragraph 2; paragraph bridging pages 233-234; page 234, full paragraph 1). PSP inhibits gastrointestinal motility and gastric acid secretion (page 231, full paragraph 1). PSP is atoxic and effective after oral administration. PSP may possess a potential utility in the treatment of gastro-duodenal ulcer diseases. Page 243, full paragraph 1. Jorgensen does not teach in the sense that Jorgensen does not anticipate a pharmaceutical composition comprising an isolated hSP polypeptide which is in N-glycosylated form and a pharmaceutically acceptable carrier.

However, it would have been obvious to one of ordinary skill in the art at the time of Applicants' invention to make an isolated hSP polypeptide which is in N-glycosylated form, as taught by Tomasetto in view of Podolsky, Alberts, Hitzeman, and Lodish, and to modify that teaching by making a pharmaceutical composition comprising an isolated hSP polypeptide which is in N-glycosylated form and a pharmaceutically acceptable carrier, as taught by Jorgensen, with a reasonable expectation of success. One of ordinary skill in the art would be motivated to make

this modification because PSP, mSP and hSP are expected to fulfill similar biological functions. The invention is prima facie obvious over the prior art.

Claims 27, 29-31, 33, 40, 60-65 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tomasetto (2, cited by Applicants) in view of Podolsky (A), Alberts (u6), Hitzeman (x6), and Lodish (w6) as applied to claims 27, 29, 30, 40, 60-62, and further in view of Onda (n6), Strausberg (C), and Gelfand (B).

Tomasetto in view of Podolsky, Alberts, Hitzeman, and Lodish teach an isolated hSP polypeptide which is in N-glycosylated form, glycosylated at an Asn present at position 15, comprising a glycosylated side chain comprising at least one hexose unit, wherein the glycosylated side chain comprises at least one mannose unit, wherein the glycosylated form comprises two units of GlcNAc. Tomasetto in view of Podolsky, Alberts, Hitzeman, and Lodish do not teach an isolated hSP polypeptide which is in N-glycosylated form, glycosylated at an Asn present at position 15, comprising a glycosylated side chain comprising at least one hexose unit, wherein the glycosylated side chain comprises at least one mannose unit, wherein the glycosylated form comprises two units of GlcNAc, wherein the glycosylated side chain comprises 13-17 mannose units, wherein the glycosylated form comprises $(GlcNAc)_2(Man)_{10-15}$.

Onda discloses the expression and secretion of a recombinant human polypeptide in E. coli, yeast cells, and animal cells (page 3, full paragraph 1; page 4, line 55; sentence bridging pages 4-5; page 5, lines 8-9 and 31-34; Example 5, pages 9-10). Onda's polypeptide has high homology with pancreatic spasmolytic polypeptide (PSP) and can be expected to fulfill similar biological functions (page 6, full paragraph 3).

The use of yeasts such as Saccharomyces as hosts for expressing mammalian and other foreign proteins offers advantages lacking in more commonly used prokaryotic hosts such as Escherichia coli. See Strausberg, column 1, full paragraph 2.

Mammalian cells are more difficult to culture than yeast. See Gelfand, column 2, lines 29-30.

Onda, Strausberg, and Gelfand do not teach an isolated hSP polypeptide which is in N-glycosylated form, glycosylated at an Asn present at position 15, comprising a glycosylated side chain comprising at least one hexose unit, wherein the glycosylated side chain comprises at least one mannose unit, wherein the glycosylated form comprises two units of GlcNAc, wherein the glycosylated side chain comprises 13-17 mannose units, wherein the glycosylated form comprises $(GlcNAc)_2(Man)_{10-15}$.

However, it would have been obvious to one of ordinary skill in the art at the time of Applicants' invention to make an isolated hSP polypeptide which is in N-glycosylated form, glycosylated at an Asn present at position 15, comprising a glycosylated side chain comprising at least one hexose unit, wherein the glycosylated side chain comprises at least one mannose unit, wherein the glycosylated form comprises two units of GlcNAc, as taught by Tomasetto in view of Podolsky, Alberts, Hitzeman, and Lodish, and to modify that teaching by expressing an hSP polypeptide in yeast with a reasonable expectation of success. One of ordinary skill in the art would be motivated to make this modification because yeasts are useful for the recombinant expression of PSP homologous peptides, yeasts offers advantages lacking in more commonly used prokaryotic hosts such as Escherichia coli, and mammalian cells are more difficult to culture than yeast. In so doing one of ordinary skill in the art would obtain an isolated hSP

polypeptide which is in N-glycosylated form, glycosylated at an Asn present at position 15, comprising a glycosylated side chain comprising at least one hexose unit, wherein the glycosylated side chain comprises at least one mannose unit, wherein the glycosylated form comprises two units of GlcNAc, wherein the glycosylated side chain comprises 13-17 mannose units, wherein the glycosylated form comprises $(GlcNAc)_2(Man)_{10-15}$ because N-linked oligosaccharides can have as many as 60 mannose residues in yeast. The invention is prima facie obvious over the prior art.

Claims 27, 42, 56, 57 are rejected under 35 U.S.C. 103(a) as being unpatentable over Tomasetto (2, cited by Applicants) in view of Podolsky (A), Alberts (u6), Hitzeman (x6), and Lodish (w6) as applied to claims 27, 42, and further in view of Potter (C).

Tomasetto in view of Podolsky, Alberts, Hitzeman, and Lodish teach an isolated hSP polypeptide which is in N-glycosylated form, glycosylated at an Asn present at position 15, comprising a glycosylated side chain comprising at least one hexose unit, wherein the glycosylated side chain comprises at least one mannose unit, wherein the glycosylated form comprises two units of GlcNAc. Tomasetto in view of Podolsky, Alberts, Hitzeman, and Lodish do not teach an isolated hSP polypeptide which is in N-glycosylated form, glycosylated at an Asn present at position 15, comprising a glycosylated side chain comprising at least one hexose unit, wherein the glycosylated side chain comprises at least one mannose unit, wherein the glycosylated form comprises two units of GlcNAc, wherein the hSP is identical to the amino acid sequence of SEQ ID NO: 1 except for the deletion of one or more amino acids at either end of SEQ ID NO: 1. However, it would have been obvious to one of ordinary skill in the art at the

time of Applicants' invention to delete one or more amino acids at either end of SEQ ID NO: 1 with a reasonable expectation of success. One of ordinary skill in the art would be motivated to make this modification because it is routine to shorten peptides at the C-terminus or at the N-terminus or at both termini in order to obtain shorter peptides having the same biological effect. See, for example, Potter column 40, penultimate paragraph. It should be understood that various changes and modifications as would be obvious to one having the ordinary skill in this art may be made without departing from the scope of the invention which is set forth in the claims appended hereto. For example, biologically active fragments of such proteins, shortened at the C-terminus or at the N-terminus or at both termini, can be employed instead of the entire protein to have the same biological effect of modulating the bioactivity CRF. The invention is prima facie obvious over the prior art.

Claim Rejections - 35 USC § 112

Claims 43-51, 53-55 rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. Support for the specific limitations in claims 44, 47, 49, 53-55 and for the combinations of limitations represented by claims 42-45, claims 42, 43, 49-51, claims 42, 43, 49, 52, and claims 42, 46-48 cannot be found in the disclosure as originally filed and the introduction of such limitations raises the issue of new matter. Applicants argue that support for added claims 42-65 is found at page 5, line 29 to page 6, line 18 (claims 42-55), at page 3, lines 24-26 (claims

56-59, and in claims 28-33 (claims 60-65). Applicant's arguments have been fully considered but they are not persuasive. The specification only supports the substitution or creation of glycosylation sites, and not any and/or all substitutions or the combinations of such limitations.

The following claims are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claim 27 recites the limitation "the nucleic acid sequence that encodes SEQ ID NO: 1." The definite article "the" denotes a single, specific nucleic acid sequence. However, due to the degeneracy of the genetic code there are an astronomical number of nucleic acid sequences encoding SEQ ID NO: 1. It is unclear which single, specific nucleic acid sequence is intended. The metes and bounds are not clearly set forth. Claims 28-33, 36, 40-65 depend from claim 27 and also share its deficiency. Claims 27-33, 36, 40-65 are rejected under 35 USC § 112, second paragraph.

Claims 43-45, 51, 52, 54 recite the limitation "first trefoil domain". There is insufficient antecedent basis for this limitation in the claims.

Claims 46-50, 55 recite the limitation "second trefoil domain". There is insufficient antecedent basis for this limitation in the claims.

Claim Objections

Claims 45, 48, 50, 51 are objected to under 37 CFR 1.75(c), as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant is

required to cancel the claim(s), or amend the claim(s) to place the claim(s) in proper dependent form, or rewrite the claim(s) in independent form. A non-substituted amino acid sequence fails to further limit a substituted amino acid sequence.

Conclusion

No claims are allowable.

ANY INQUIRY CONCERNING THIS COMMUNICATION OR EARLIER COMMUNICATIONS FROM THE EXAMINER SHOULD BE DIRECTED TO DAVID S. ROMEO WHOSE TELEPHONE NUMBER IS (571) 272-0890. THE EXAMINER CAN NORMALLY BE REACHED ON MONDAY THROUGH FRIDAY FROM 7:30 A.M. TO 4:00 P.M. IF ATTEMPTS TO REACH THE EXAMINER BY TELEPHONE ARE UNSUCCESSFUL, THE EXAMINER'S SUPERVISOR, GARY KUNZ, CAN BE REACHED ON (571) 272-0887.

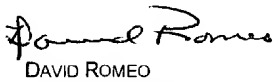
IF SUBMITTING OFFICIAL CORRESPONDENCE BY FAX, APPLICANTS ARE ENCOURAGED TO SUBMIT OFFICIAL CORRESPONDENCE TO THE FOLLOWING TC 1600 BEFORE AND AFTER FINAL RIGHTFAX NUMBERS:

BEFORE FINAL (703) 872-9306
AFTER FINAL (703) 872-9307

CUSTOMERS ARE ALSO ADVISED TO USE CERTIFICATE OF FACSIMILE PROCEDURES WHEN SUBMITTING A REPLY TO A NON-FINAL OR FINAL OFFICE ACTION BY FACSIMILE (SEE 37 CFR 1.6 AND 1.8).

FAXED DRAFT OR INFORMAL COMMUNICATIONS SHOULD BE DIRECTED TO THE EXAMINER AT (571) 273-0890.

ANY INQUIRY OF A GENERAL NATURE OR RELATING TO THE STATUS OF THIS APPLICATION OR PROCEEDING SHOULD BE DIRECTED TO THE GROUP RECEPTIONIST WHOSE TELEPHONE NUMBER IS (703) 308-0196.



DAVID ROMEO
PRIMARY EXAMINER
ART UNIT 1647

DSR
APRIL 8, 2004

Notice of References Cited	Application/Control No. 09/528,644	Applicant(s)/Patent Under Reexamination THIM ET AL.	
	Examiner David S Romeo	Art Unit 1647	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-6,063,755	05-2000	Podolsky, Daniel K.	514/2
*	B	US-4,870,013	09-1989	Gelfand et al.	435/69.1
*	C	US-5,013,652	05-1991	Strausberg et al.	435/69.2
*	D	US-5,464,757	11-1995	Potter et al.	435/69.1
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
√	U	Jorgensen et al. Pancreatic spasmolytic polypeptide (PSP): III. Pharmacology of a new porcine pancreatic polypeptide with spasmolytic and gastric acid secretion inhibitoryeffects. Regul Pept. 1982 Mar;3(3-4):231-43.
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Regulatory Peptides, 3 (1982) 231–243
Elsevier Biomedical Press

Pancreatic Spasmolytic Polypeptide (PSP):

III. Pharmacology of a new porcine pancreatic polypeptide with spasmolytic and gastric acid secretion inhibitory effects

[1] Karin Damm Jørgensen, [1] Bertil Diamant, [2] Klavs H. Jørgensen and [2] Lars Thim

[1] *Laboratory of Experimental Pharmacology, Pharmaceuticals R&D, and* [2] *Laboratory of Protein Chemistry, NOVO Research Institute, Novo Allé, DK-2880 Bagsvaerd, Denmark*

(Received 27 July 1981; revised manuscript received 8 October 1981; accepted 13 October 1981)

Summary

Pancreatic spasmolytic Polypeptide (PSP) is a new porcine pancreatic polypeptide, which inhibits gastrointestinal motility and gastric acid secretion in laboratory animals after parenteral as well as oral administration.

(1) PSP inhibits the amplitude of electrically stimulated contractions of the isolated guinea pig ileum. PSP's inhibitory effect is antagonized by phentolamine, but not by yohimbine.
(2) PSP inhibits the motility of isolated guinea pig intestinal segments after intraluminal dosing.
(3) PSP reduces intestinal motility in rabbits in vivo after intravenous and intraluminal administration, and in mice in vivo after subcutaneous injection.
(4) PSP delays absorption of protein hydrolysate when it is administered orally in capsules to pigs and to pancreatectomized dogs.
(5) PSP inhibits pentagastrin induced gastric acid secretion in rats after oral administration and in cats after subcutaneous and oral administration.

The mechanism of action of PSP has so far not been finally elucidated. It seems likely that PSP interferes with endogenous acetylcholine release. Furthermore it might act by release of somatostatin from somatostatin cells in the gastrointestinal tract. It may have a direct or an indirect stimulant effect on α_1-receptors.

gut; motility; absorption; gastric acid secretion; pentagastrin; somatostatin; α-receptors

Introducti n

Pancreatic Spasmolytic Polypeptide (PSP) is a new porcine pancreatic polypeptide which has been chemically characterized by Jørgensen et al. [4]. Thim et al. [7] established a RIA for PSP and found that the peptide is restricted to the pancreas and that it is present in the exocrine pancreas secretion. The aim of the present studies was to evaluate the pharmacological effects of PSP, isolated and purified by Klavs H. Jørgensen and Lars Thim.

Materials and Methods

Spasmolytic effects in vitro

Studies on the electrically stimulated guinea pig ileum. The method was a modification of that described by Day et al. [2]. Six guinea pigs of either sex, weighing 300–350 g, were killed by a blow to the head followed by exsanguination, and segments of ileum orally of the ampulla and 1.5–2 cm long were mounted in an organ bath containing 15 ml Tyrode solution, kept at 37°C, and bubbled with 95% O_2 and 5% CO_2, under a tension of 1 g. Changes in tension were recorded by an absorbance wedge and a Devices photoelectric transducer connected to a Kipp & Zonen recorder. Submaximal twitch responses were obtained by transmural stimulation at 1–5 V using platinum electrodes and a Grass stimulator. The pulse rate was 0.2–0.3 pps and the duration of the pulses was 50 ms. The peptides were dissolved in 0.9% saline with 0.1% human serum albumin (HSA). Volumes of 100 µl were added to obtain the final concentrations in the bath. The data were statistically analysed by an analysis of variance in a parallel line assay.

Studies on guinea pig intestinal segments. Segments of ileum, jejunum, and duodenum from nine guinea pigs were mounted in an organ bath as described above with the following modification: The segments, which were 1.5 cm long, were closed with a silk suture in the aboral end. In the oral end they were closed around an Intramedic® polyethylene catheter, PE 50, which was connected with a syringe for intraluminal dosing. The intraluminal dosing with 0.1 ml placebo (physiological saline with 0.1% HSA) would induce a motility which would last for at least four to five minutes, and which could be reproduced. In this way drugs could be screened for inhibitory effect on the motility after intraluminal dosing.

Spasmolytic effects in vivo

Experiments in anaesthetized rabbits. Ten Rex rabbits of either sex, weighing 2.5–3.0 kg, were anaesthetized by intravenous injection of pentobarbital (30 mg/kg) via an ear cannula, and laparotomy was performed. Balloon catheters were placed in duodenum, jejunum or colon, filled with saline and connected to a Statham physiological pressure transducer. Recordings were obtained using a Devices re-

corder. For the intraluminal dosing of test compounds a catheter was placed with its tip approximately 2 cm orally of the balloon. Both catheters were inserted into the intestine through a small opening 20 cm orally of the final position of the balloon. The opening was closed around the catheters with a tobacco pouch suture.

Effects on gastrointestinal charcoal propulsion in mice. 120 female NMRI mice of 20 ± 2 g were fasted for 18 h. The test substances were administered subcutaneously 15 min before oral administration of a charcoal suspension to groups of 10 mice. 30 min after dosing with the charcoal suspension the animals were killed with CO_2 and the extent of charcoal propulsion in the small intestine was measured. The inhibiting activity of the test substances on the intestinal transport was determined graphically as the ED_{50}, i.e the dose, causing a 50% inhibition of propulsion in comparison with the placebo group. The relative potencies were determined according to a method described by Litchfield and Wilcoxon [5].

Effect on the absorption of protein hydrolysate in pigs and in pancreatectomized dogs

Two female pigs from the same litter, DLR/Yorkshire breed, weighing about 30 kg, and two totally pancreatectomized mongrel dogs of either sex, weighing 25–30 kg, were used in the studies, which were carried out as crossover studies. The animals were fasted overnight. Immediately after oral dosing with a capsule with 3 mg PSP or placebo (sodium chloride), protein hydrolysate was administered through stomach tubes. 100 μCi [U-^{14}C]protein hydrolysate was mixed with a suspension of 1 g/kg Idon® in 250 ml water. Blood samples were taken into heparinized glasses from an ear vein in the pigs and from a saphenous vein in the dogs, at −5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 105, 120, 150, 180, 240, 300, and 360 min. To 1 ml plasma were added 10 ml Instagel®, and plasma dpm values were determined by means of a Rack Beta liquid scintillation counter (LKB). The data after placebo and PSP, respectively, were compared using two-way analyses of variance.

Effects on gastric acid secretion

Effect on pentagastrin induced gastric acid secretion in rats in vivo. Three male Wistar rats, weighing 150–160 g, were anaesthetized with pentobarbital, 50 mg/kg i.p. The fur on the abdomen was shaved, and a midline incision was made. A gastric fistula made of plexiglas ad modum P. Kirkegaard (personal communication) was inserted into the stomach in the grey part. The fistula was led through the abdominal wall 0.5 cm left of the midline. The wound was closed with silk sutures, and the fistula was closed with an aluminum cap. The rats were allowed 14 days to recover after the operation before they were used in the test. Prior to the test the rats were fasted overnight in single cages of double wire mesh to prevent coprophagia. During the test the rats were placed in restraint cages to which they had previously become accustomed. The fistulas were rinsed mechanically and washed with redestilled water. After 20 min a collection of gastric secretion was initiated. Collection was made over periods of 30 min. 10 μg pentagastrin (Peptavlon® diluted with 0.9% saline with 0.1% HSA to a volume of 1 ml) was administered subcutaneously at 60

min. The study was performed as a crossover experiment and the test substances were administered orally in 1 ml saline with 0.1% HSA via ventricular tubes at 45 min. The fistulas were closed with forceps during the administration. PSP was compared to placebo and to somatostatin. The collection of gastric juice continued for 2 h after pentagastrin dosing. The volumes were recorded and the acidity determined by titration with 0.01 N NaOH solution, using phenolphthalein as an indicator. Increments over basal acid secretion for each time period, and sum of the observations were compared using two way analyses of variance. The secretion during the first period was used as the basal secretion, as the secretion during the second period was influenced by the stress caused by the oral dosing at 45 minutes.

Effect on pentagastrin induced gastric acid secretion in cats in vivo. Six cats of either sex, weighing 2.5–4.5 kg, were prepared with chronic gastric fistulas.

The fistulas were placed in the fundus ventriculi in a way so that they were situated in the lowest part of the ventricle, when the cats were lying on their left side to allow a nearly quantitative collection by gravity of gastric secretion. The cats were fasted overnight prior to the experiments. On the day of the experiment the fistula was rinsed mechanically and washed with redestilled water. After 20 min a collection of gastric secretion was initiated. Collection was made over periods of 15 min. and after four periods, pentagastrin (Peptavlon®) was administered subcutaneously in a dose of 1 μg/kg. Capsules with placebo or 250 μg PSP were given orally at 55 and 100 min. The collection of gastric juice continued for 1.5 h after pentagastrin dosing. The volumes and pH were recorded, and the acidity determined by titration with 0.01 N NaOH solution using phenolphthalein as an indicator. The six cats were dosed with placebo and with PSP with a varying number of repetitions. Increments over basal acid secretion for each of the time periods, and sum and maximum of the observations were compared using two-way analyses of variance.

Materials

PSP was purified as previously described [4]. Glucagon used in the studies was NOVO porcine glucagon, synthetic somatostatin was purchased from Peninsula Laboratories, Inc. Phentolamine hydrochloride was from Ciba-Geigy, and yohimbine hydrochloride from Sigma. Human serum albumin was from Behringwerke. Peptavlon® was from ICI, ±-propranolol hydrochloride from Sigma, and naloxone from Endo. [U-^{14}C]Protein hydrolysate, [2-^{3}H]glucose and [U-^{14}C]ovalbumin was obtained from Amersham.

Experimental results

Spasmolytic effects in vitro

Studies on the electrically stimulated guinea-pig ileum

PSP (10^{-8} to 10^{-4} M) inhibited in a dose-dependent way the amplitude of electrically evoked contractions of the guinea pig ileum. The effect developed from

15–30 s after the addition to the organ bath to reach a maximum after 1 min. Th duration of the effect was dose-dependent, and the effect was revers d after washout (Fig. 1.)

The effects of PSP alone and in the presence of 10^{-9} M phentolamine and the effects of somatostatin were compared on three ileum preparations (from three

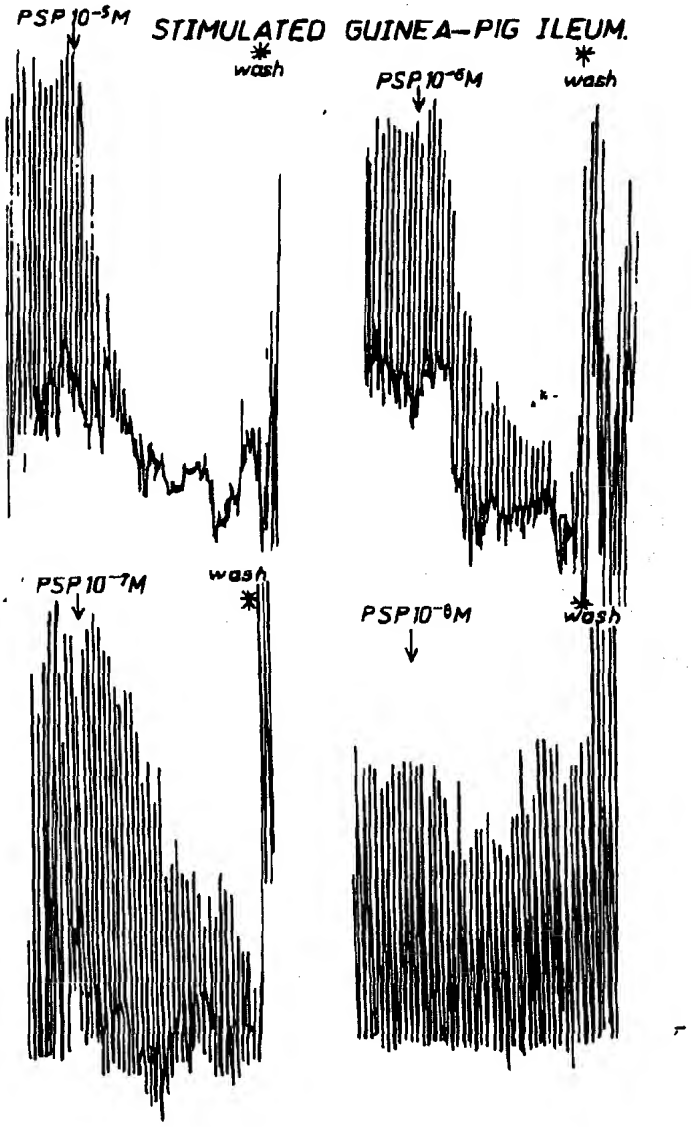


Fig. 1. Tracing illustrating the inhibitory effects of different concentrations of PSP on electrically evoked contractions of the guinea pig ileum. PSP was left in contact with the tissue for two minutes before wash.

guinea pigs), and dose response curves were drawn by eye (Fig. 2). The ID_{50}, i.e. the doses required to inhibit the contractions to 50% of control, read off the curves, was for PSP approximately $1.2 \cdot 10^{-6}$ M, for PSP in the presence of phentolamine about $6 \cdot 10^{-6}$ M, and for somatostatin about $2.5 \cdot 10^{-5}$ M.

Based on the values between 20 and 80% inhibition the relative potencies were calculated in a parallel line assay. PSP was found to possess 4.4% of somatostatin's activity (95% confidence limits being 2.5–7.4%). PSP alone had 7.5 times the activity of PSP in the presence of 10^{-9} M phentolamine, the confidence limits being 4.5–12.0 times.

The results indicate that the inhibitory action of PSP was competitively antagonized by the previous addition of phentolamine. In contrast, yohimbine, propranolol and naloxone did not modify the effect of PSP (not shown).

The effects of increasing concentrations of somatostatin alone and in the presence of 10^{-7} M phentolamine were compared on three other preparations (results not shown). The ID_{50} was found to be approximately 10^{-7} M for somatostatin alone and 10^{-5} M for somatostatin in the presence of phentolamine.

Studies on guinea pig intestinal segments

PSP which has been treated with trypsin, chymotrypsin, and hydrochloric acid, as described by Jørgensen et al. [4], maintained full biological activity in the stimulated guinea pig ileum assay. On this background it was decided to test whether PSP was able to inhibit the motility after intraluminal dosing to isolated intestinal segments.

PSP had a marked inhibitory effect on the motility of segments of guinea pig ileum, duodenum, and jejunum in doses from 5 to 500 μg administered intralumi-

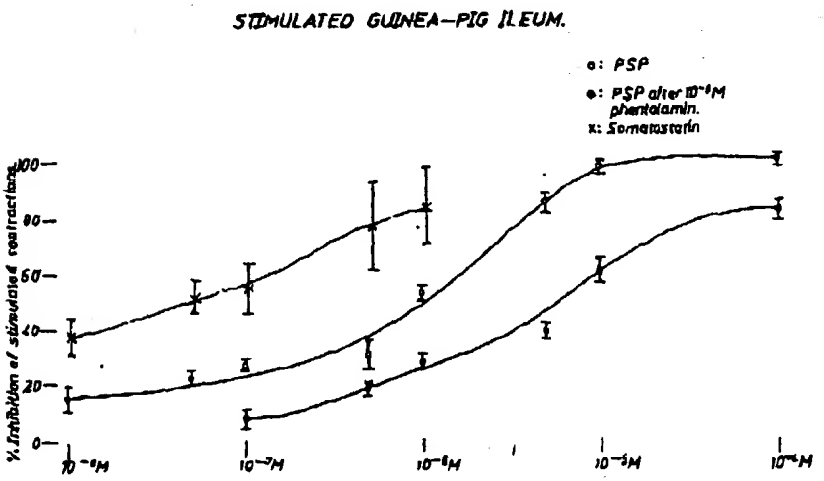


Fig. 2. Dose–response curves obtained with PSP, PSP in the presence of phentolamine and somatostatin on the electrically stimulated guinea pig ileum. Each point is the mean±S.D. for 3 determinations. Phentolamine was added to the organ bath 2 min before the addition of PSP.

nally in 0.1 ml volumes of 0.9% saline with 0.1% human serum albumin. The effect is illustrated in Fig. 3. Sometimes the higher doses of PSP caused a complete relaxation (decrease in tonus) of the intestine which was reflected in a shift of the baseline. The duration of the effect was 3–5 min.

Spasmolytic effects in vivo

Experiments in rabbits

PSP reduced the intestinal motility in rabbits in vivo as registered by the pressure on a balloon catheter in the lumen of various parts of the intestine: duodenum, jejunum, and colon. The inhibitory effect was seen both after intravenous bolus injection of PSP and after local administration into the lumen of the intestine. In five out of five rabbits the administration of 150 μg/kg PSP intravenously or intraluminally caused a strong reduction of the motility till nearly atonia of the intestine (Fig. 4). The effect started 30–60 s after the dosing and lasted for 3–10 min. In three out of five rabbits 75 μg/kg PSP had a distinct effect. Glucagon was used as a reference peptide in this test, and it was found that glucagon inhibited the intestinal motility to the same extent after intravenous bolus administration of 75–150 μg/kg, i.e. approximately three times as high doses on a molar basis. Contrary to PSP, glucagon had no effect after intraluminal dosing.

Effects on gastrointestinal charcoal propulsion in mice

The inhibiting activity of PSP, glucagon, and atropine sulphate upon charcoal propulsion in the small intestine of mice is summarized in Table I. PSP delayed the intestinal transport after subcutaneous administration. On the basis of the ED_{50}

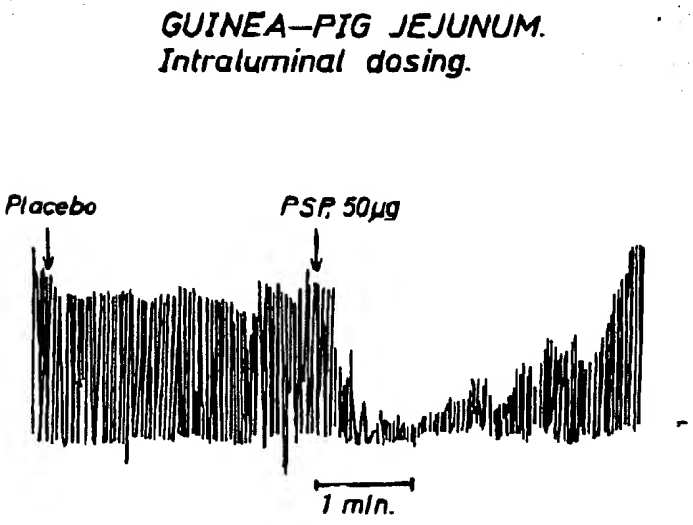


Fig. 3. Tracing illustrating the inhibitory effect on the motility of guinea pig jejunum in vitro of 50 μg PSP administered intraluminally in a volume of 0.1 ml.

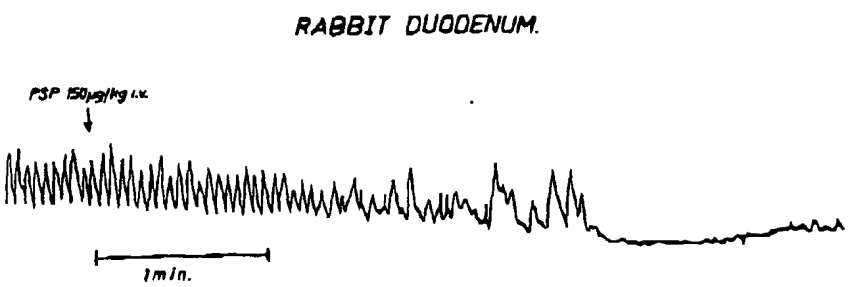


Fig. 4. Tracing illustrating the inhibitory effect on duodenal motility in a rabbit in vivo of 150 μg/kg PSP administered intravenously as a bolus.

values it appears that PSP has 1.5 times the potency of glucagon and 6.4 times the potency of atropin in the test. The dose response curves were found to be parallel. PSP was significantly more active than atropin, and for 95% confidence limits its relative molar potency was between 1.5 and 27.0 times that of atropin. The corresponding value for PSP compared to glucagon was found to be between 0.5 and 5.0 on a molar basis.

Effect on the absorption of protein hydrolysate in pigs and in pancreatectomized dogs

In the pigs, which were dosed with 100 μCi [U-^{14}C]protein hydrolysate mixed with Idon® (1 g/kg), the plasma dpm values were significantly lower after oral administration of 3 mg PSP than after placebo (Fig. 5) for all times tested ($p < 0.01$ for 240 and 300 min, $p < 0.001$ for all other times). Moreover, maximal plasma dpm values were obtained markedly earlier after placebo than after PSP.

In the pancreatectomized dogs which were given 100 μCi [U-^{14}C]protein hydrolysate mixed with Idon®, the absorption after 3 mg PSP was delayed, compared to placebo. Plasma dpm were significantly lower at 10 and 20 min after PSP dosing as compared to placebo dosing ($p < 0.001$). However, the curves crossed each other at 70 min (Fig. 6). When the dogs were given additional capsules with 3 mg PSP at 25 and 50 min, the absorption of the protein hydrolysate was further delayed, and the curves did not cross until 360 min.

TABLE I

Effect on gastrointestinal propulsion in mice

Drug *	ED_{50} with 95% confidence limits (μmol/kg)
PSP	13 (6– 30)
Glucagon	20 (9– 46)
Atropine	83 (26–272)

* All drugs were administered by the subcutaneous route.

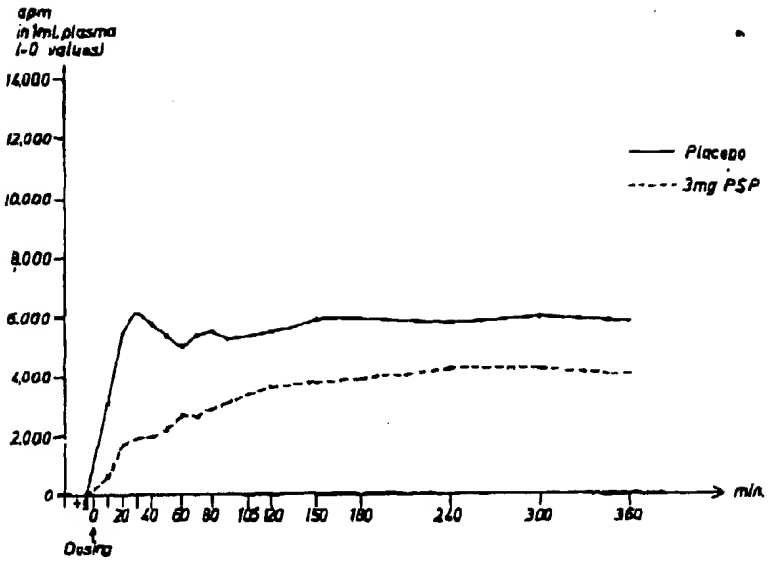


Fig. 5. Plasma dpm values after oral administration to pigs of 1 g/kg Idon® with 100 μCi [U-^{14}C]protein hydrolysate immediately after oral dosing with a capsule with placebo or PSP. The curves represent the mean values for two pigs.

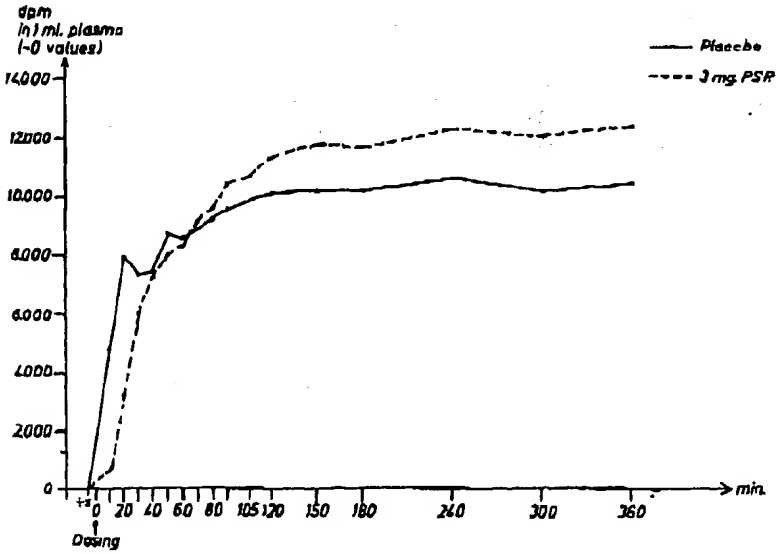


Fig. 6. Plasma dpm values after oral administration to pancreatectomized dogs of 1 g/kg Idon® with 100 μCi [U-^{14}C]protein hydrolysate immediately after oral dosing with a capsule with placebo or PSP. The curves represent the mean values for two dogs.

Effects on gastric acid secretion

Effect on pentagastrin induced gastric acid secretion in rats in vivo

Basal gastric acid secretion per 30 min varied from 3–42 μequiv. acid. The stress, inflicted to the rats by the oral dosing was reflected in an increase in acid secretion in all animals (Fig. 7). The subcutaneous administration of 10 μg pentagastrin induced a peak increment of 80 μequiv. acid during the first 30-min period after the administration. For this period the acid output was significantly lower ($p < 0.05$) after oral dosing with PSP, 100 μg ($8.9 \cdot 10^{-9}$ mol) and after oral dosing with somatostatin, 100 μg ($6.1 \cdot 10^{-8}$ mol) ($p < 0.01$). The secretion after somatostatin was significantly lower than after PSP ($p < 0.05$).

Effect on pentagastrin induced gastric acid secretion in cats in vivo

Basal gastric acid secretion was approximately 10-20 μequiv. acid per 15-min period. The subcutaneous administration of 1 μg/kg pentagastrin induced a peak increment of 377 ± 45 (mean ± S.D.) μequiv. acid during the second period, i.e. from 15 to 30 min after the administration. The oral dosing with PSP reduced acid output significantly ($p < 0.01$) to 79 ± 60 μequiv. acid during the same period (Fig. 8). Besides the acid secretion from 45 to 60 min after pentagastrin was lower after PSP ($p < 0.05$). When sum and maximum values were compared for placebo and PSP, it

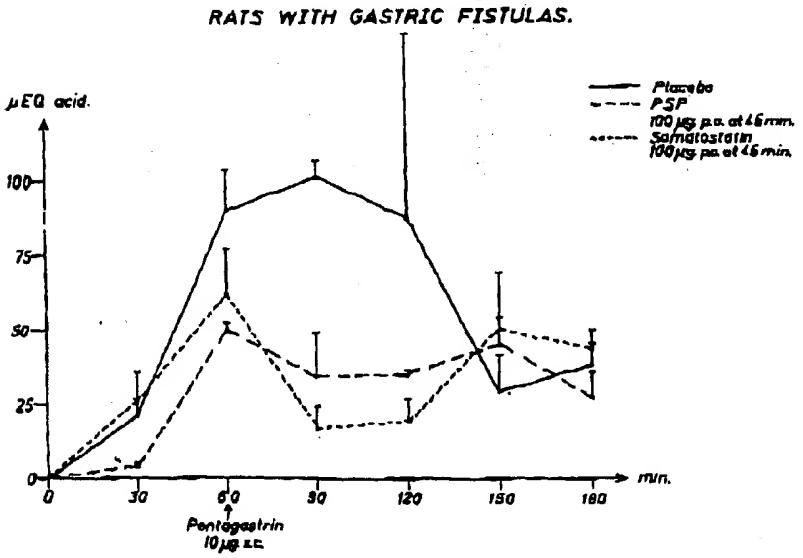


Fig. 7. Gastric acid secretion in rats after pentagastrin stimulation. Collection of gastric secretion was made over periods of 30 min. 100 μg PSP administered orally at 45 min inhibited the acid secretion during the period from 60 to 90 min ($p < 0.05$). 100 μg somatostatin inhibited the acid secretion during the same period ($p < 0.01$). Each point is the mean ± S.E.M. for three rats.

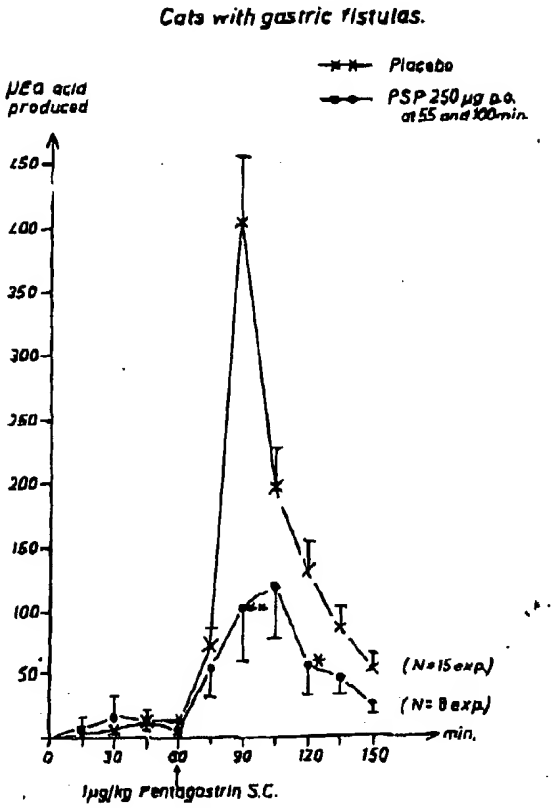


Fig. 8. Gastric acid secretion in cats after pentagastrin stimulation. Collection of gastric secretion was made over periods of 15 min. 250 µg PSP administered in a capsule at 55 and 100 min inhibited the acid secretion during the period 75–90 minutes ($p<0.01$) and the period 105–120 minutes ($p<0.05$). Each point is the mean ± S.E.M.

was likewise found that the acid output was significantly lower after PSP treatment ($p<0.01$). 250 µg PSP (approximately 70 µg/kg body weight) administered twice, at 55 and 100 min in capsules p.o. to cats caused a 64% inhibition of acid secretion.

For comparison was noticed that 10 µg/kg PSP administered twice at 60 and 105 min subcutaneously to three gastric fistula cats caused an average inhibition of 64% of the acid secretion induced by 1 µg/kg pentagastrin administered subcutaneously. In two cats the gastric acid secretion after 2 µg/kg pentagastrin administered subcutaneously was inhibited 55% by 20 µg/kg ($1.8 \cdot 10^{-9}$ mol/kg) PSP subcutaneously and 43% by 2 µg/kg ($1.2 \cdot 10^{-9}$ mol/kg) somatostatin subcutaneously.

Discussion

As PSP is present in the porcine pancreas, it was decided to initiate the pharmacological screening by studies of metabolic and gastrointestinal effects. The

results reported in this paper indicate that PSP inhibits gastrointestinal motility and gastric acid secretion after parenteral as well as oral administration.

PSP did not modify contractions of the guinea-pig ileum caused by direct activation of muscarinic receptors with carbachol. The electric stimulation of the ileum causes a release of endogenous acetylcholine [1]. This release leads to contractions which as found by us and others [1] can be inhibited by atropin and hexamethonium. It seems likely that PSP causes an inhibition of the release of endogenous acetylcholine in the electrically stimulated ileum, either at the ganglionic or the peripheral level.

The results further indicate an involvement of α_1-receptors in the inhibitory effect of PSP. The finding that yohimbine, propranolol, and naloxone did not modify the effect of PSP seems to exclude α_2-, β-, and opiate receptors in the mechanism of action of PSP. Furthermore, the effect of somatostatin has similarities with the effect of PSP in our test model: while phentolamine antagonized the effect of both agents yohimbine, propranolol, and naloxone did not. Furness and Costa [3] found an inhibitory effect of somatostatin on contractions caused by electric stimulation of guinea pig ileum and reported that this effect was not influenced by naloxone. However, contrary to our results, they found that phentolamine did not antagonize the effect of somatostatin. An additional mechanism of action of PSP could be a somatostatin releasing effect on the somatostatin cells in the gastrointestinal tract, and preliminary results have shown an increase in the somatostatin content in the Tyrode solution after addition of PSP to the bath containing the stimulated ileum. No increase occurred after placebo.

Not only the inhibitory effect of PSP on the gastrointestinal motility but also its inhibitory action on gastric acid secretion can be mimicked by somatostatin [6]. However, if the main action of PSP would be mediated by release of somatostatin, PSP does not release somatostatin from all somatostatin cells. Thus, no somatostatin release was seen after infusion of PSP into the isolated perfused rat pancreas at concentrations up to 10^{-5} M, and PSP had no metabolic effects as determined by changes in plasma glucose and IRI either in normal rats in vivo or in glucose primed or streptozotocin diabetic rats. Contrary to somatostatin [6] PSP was also found to be devoid of effects on the exocrine pancreatic secretion (results not shown).

Apparently, PSP causes a delay in the absorption of various compounds. The protein hydrolysate used in the absorption test was composed essentially of free amino acids only, which are readily absorbed through the mucosa. Moreover, PSP was found to delay the absorption of orally administered [2-^{3}H]glucose in pigs and in pancreatectomized dogs and of [U-^{14}C]ovalbumin in pancreatectomized dogs (results not shown). It does not seem likely that PSP can have a direct inhibitory effect on the absorption of both amino acids and glucose. Besides, preliminary experiments have indicated that PSP is no protease inhibitor [4]. However, the delay in absorption may be attributable to an inhibition of gastrointestinal motility, leading to a decreased contact of the gastric contents with the mucosal surface and a delayed passage through the gastrointestinal tract.

The pharmacological screening of PSP comprised further screening for CNS effects (acute toxicity with observations, animex and rotarod tests, potentiation of

narcosis), screening for cardiovascular effects, and effects on diuresis, the screening for effects on neuromuscular transmission, effects on bile flow and external pancreas secretion, and effect on thrombocyte aggregation; but the peptide was found to be inactive in all these tests (results not shown).

PSP has been found to be a widely atoxic drug. Besides, the peptide is effective after oral administration. If the results in animal experiments can be confirmed in man, PSP may possess a potential utility in the treatment of gastroduodenal ulcer diseases.

Acknowledgements

We wish to thank P.C. Pedersen for carrying out the analyses of variance and Mrs. L.S. Neil for typing the manuscript. The skillful technical assistance of Bengt Pedersen, Aase R. Kofod, Torben R. Schou, Ingeborg M. Peippo, Birthe Frederiksen, and Elke Gottfriedsen is gratefully acknowledged.

References

1 Cowie, A.L., Kosterlitz, H.W., and Waterfield, A.A., Factors influencing the release of acetylcholine from the myenteric plexus of the ileum of the guinea-pig rabbit. Br. J. Pharmacol. 64 (1978) 565–580.
2 Day, A.R., Luján, M., Dewey, W.L., Harris, L.S., Radding, J.A., and Freer, R.J., Structure activity relationships of enkephalins in the stimulated guinea pig ileum. Res. Commun. Chem. Pathol. Pharmacol. 14 (1976) 597–603.
3 Furness, J.B. and Costa, M., Actions of somatostatin on excitatory and inhibitory nerves in the intestine, Eur. J. Pharmacol., 56 (1979) 69–74.
4 Jørgensen, K.H., Thim, L., and Jacobsen, H.E., Pancreatic Spasmolytic Polypeptide (PSP). I. Preparation and chemical characterization of a new polypeptide from porcine pancreas. Regul. Peptides. 3 (1982) 207–219.
5 Litchfield, J.T. and Wilcoxon, F., A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther., 96 (1949) 99–113.
6 McQuillan, M.T., Somatostatin, In D.F. Horrobin (Ed.), Annual Research Reviews. Vol. 2, pp. 36–51, 1980. Eden Press., St. Albans, VT, U.S.A.
7 Thim, L., Jørgensen, K.H., and Jørgensen, K.D., Pancreatic Spasmolytic Polypeptide (PSP). II. Radioimmunological determination of PSP in porcine tissues, plasma and pancreatic juice. Regul. Peptides, 3 (1982) 221–230.